AN ABSTRACT OF THE THESIS OF

Sharad B. Murdande for the degree of Master of Science in Pharmaceutics presented on 5 th January 1994.

Title: The Disposition of Four Therapeutically Important Antimicrobial Agents in Llamas

Abstract approved: Redacted for Privacy

Dr. J. Mark Christensen

The disposition of four therapeutically important antimicrobial agents was studied in llamas following intravenous bolus administration. Six llamas were each given ampicillin, tobramycin, trimethoprim and enrofloxacin at a dose of 12 mg/kg, 1 mg/kg, 3 mg/kg and 5 mg/kg of body weight with a wash out period of 3 days between each treatment. Plasma concentrations of these antimicrobial agents over 12 hours following IV bolus dosing were determined by reverse phase HPLC. Dispositions of these four antimicrobial agents were described by two compartment open model with elimination from the central compartment, and also by non-compartmental methods. From compartmental analysis, the elimination rate constant, half-life, and apparent volume of distribution in the central compartment were determined. Statistical moment theory was used to determine non-compartmental pharmacokinetic parameters of mean residence

time, clearance, and volume of distribution at steady-state. Based on the disposition parameters determined, a dose and dosing interval for each of the four antimicrobial agents was suggested for llamas. Steady state peak and trough plasma levels were also predicted for the drugs in this study for llamas.

The Disposition of Four Therapeutically Important Antimicrobial Agents in Llamas

by

Sharad B. Murdande

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Completed 5 th January 1994

Commencement June 1994

Approved:

Redacted for Privacy

Associate Professor of Pharmaceutics in charge of major

Redacted for Privacy

Dean of Pharmacy School

Redacted for Privacy

Dean of Graduate School

Date thesis is presented 5th January 1994.

Thesis of: Sharad B. Murdande

Acknowledgement

I take this opportunity to express my gratitude to Dr. J. Mark Christensen, Associate Professor, College of Pharmacy, Oregon State University, under whose supervision, invaluable guidance and constant encouragement this work was materialized.

I thank Dr. Brad Smith, Associate Professor, Veterinary Medicine, for providing the financial support. My thanks to Dr. J. W. Ayres and Dr. S. W. Hoag for serving on my graduate committee.

My special thanks are due to Dr. Cliff Pereira, Department of Statistics, for acting as minor professor on my committee.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (CONTINUED)

THE DISPOSITION OF FOUR THERAPEUTICALLY IMPORTANT ANTIMICROBIAL AGENTS IN LLAMAS

INTRODUCTION

Bacterial infections of the reproductive, gastrointestinal, and respiratory systems are common problems in llamas (1,2,3). Due to the behavioral characteristics of llamas, bacterial infections and pathologic changes are frequently severe before clinical changes are noted, making appropriate antibiotic selection and dosing of paramount importance. Since the clearance characteristics of antibiotics have not been established for many drugs in the llama, drug dosages and intervals have been extrapolated from other species. While drug dosages extrapolated from related species (e.g., dog to wolf) are frequently sufficiently accurate for treatment of common bacterial infections, extrapolation across genera and family lines is frequently inappropriate. This problem is particularly acute for the South American Camelids (llama, alpaca, guanaco and vicuna). Since few drug's dosages have been established for their closest phylogenetic relatives, the bactrian and dromedary camels, drug dosages are frequently extrapolated from those known for the cow, sheep and goat species distantly phylogenetically related to the South American Camelids.

Species differences in dosage or administration rate (dose/dosing interval) may be attributed to variations in pharmacokinetic behavior or

pharmacodynamic activity or both. Comparative pharmacokinetic studies help to explain differences in absorption and disposition processes between species in response to fixed dosages of a drug (4). Species variations in pharmacokinetic behavior of a drug in mammals are usually attributed to differences in the rate of elimination rather than distribution and metabolism of the drug, although the principle metabolic pathways may differ (4). The half-life values of drugs that undergo extensive hepatic metabolism vary widely among the species of domestic animals and humans. With certain notable exceptions, the herbivorous species (horses and ruminant animals) metabolize lipid-soluble drugs more rapidly than carnivorous species (dogs and cats). Humans metabolize drugs slowly in comparison with animals, and generally have longer half-life values (4).

Inter species comparison of pharmacokinetic parameters between other orders of animals (e.g., birds, fish, reptiles) show that variations may be extreme. This can occur even when the principle elimination mechanism for the drug is renal excretion (5). Most species differences in pharmacological effects after a fixed dosage of a drug are due to variations in pharmacokinetic characteristics of the drug, principally the rate of drug microsomal metabolism (oxidative reactions and glucuronide synthesis) (4). These differences can generally be accommodated by adjusting the dosage interval.

Comparative pharmacokinetic studies provide a technique to clarify differences in absorption and disposition processes between species in response to a fixed dosage of a drug.

The number of llamas in North America are steadily increasing with total population estimates varying between 40,000 and 60,000 animals. In light of their high value and increasing popularity, it is appropriate to establish normal drug distribution, disposition and elimination processes for representative antimicrobial drugs in llamas. Antimicrobial drug dosages in the llama and alpaca are frequently based on clearance characteristics determined in sheep due to the similarity in weight and nutrient requirements. This approach overlooks the fact that the Tylopoda (camel family) and the Bovidae (sheep and cattle) diverged phylogenetically over 55 million years ago (6). It is also assumed that since the Bovidae and Tylopoda have similar grazing habits and common species of commensal bacteria and protozoa, that intestinal drug absorption is handled in the same manner. This assumption is probably unwarranted in at least some situations since the Bovidae is a four compartment animal gastro-intestinally while the Camelids have a total of three compartments with only the distal 20% of the third compartment being the functional equivalent of the abomasum. There is also good evidence that the absorptive capabilities of the large first compartment (C-1) are significantly different than the rumen of the cow and sheep (3).

When drug dosages are extrapolated across species line, the assumption being made is that the pharmacokinetic parameters remain constant. As illustrated in Table 1-4, these assumptions are frequently invalid resulting in under or over dosing of a pharmaceutical. To date there have been no critical pharmacokinetic evaluations of antimicrobial drugs for use in llamas to verify if extrapolation of drug dosages from other species is appropriate.

Table 1. Species comparison of pharmacokinetic values of Enrofloxacin for poultry, calves, rabbits and fish after a single IV bolus dose (7-10).

Para-meter	Units	Turkey	Chicken	Calf	Rabbit	Fish
$t_{1/2}$	h	4.1	18.7	2.7	2.5	24.4
CL	ml/h/kg	532	134	204	606	91.6
V_{darea}	l/kg	3.16	3.61	0.77	2.12	3.22
AUC	μg.h/ml	18.82	37.3	26.7	8.6	109.2

Table 2. Species comparison of pharmacokinetic values of Ampicillin for sheep, mice, horse and humans after a single IV bolus dose (11-14).

Para meter	Units	Sheep	Mice	Horse	Human
$t_{1/2}$	h	0.79	0.96	1.6	1.09
CL	ml/h/kg	327.6	360.0	----	216.2
V_{darea}	l/kg	0.52	0.38	----	0.34
AUC	μg.h/ml	34.51	111.37	----	55.8

Table 3. Species comparison of pharmacokinetic values of Tobramycin for cats and humans after a single IV bolus dose (15-16).

Parameter	Units	Cat	Human
$t_{1/2}$	h	1.84	1.59
CL	ml/h/kg	132.6	105.0
V_{darea}	l/kg	0.20	0.241
AUC	μg.h/ml	8.16	----

Table 4. Species comparison of pharmacokinetic values of Trimethoprim for rats and horse after a single IV bolus dose (17,18,49).

Parameter	Units	Rat	Horse	Human
$t_{1/2}$	h	1.65	3.92	14.6
CL	ml/h/kg	3120	72	----
V_{darea}	l/kg	5.73	0.39	----
AUC	μg.h/ml	1.25	201.07	----

Establishment of appropriate therapeutic regimens requires determination of a drug's half-life, clearance and volume of distribution (19). Once these parameters have been established, drug dosing can be established in order to maintain a minimum inhibitory concentration (MIC) of the pharmaceutical. The lack of information about drug disposition in llamas led to

this study, which was to evaluate the pharmacokinetics of four commonly used antimicrobials from four separate classes of drugs. The results of this study will begin to provide a rational basis for therapeutic treatment of infectious diseases in llamas. By testing the antimicrobials in llamas, and determining their pharmacokinetic parameters, the probability of developing appropriate drug dosing schedules should significantly increase and greatly aid in therapy. Developing drug dosing schedules from this study may aid veterinary practitioners in their attempt to extrapolate other antimicrobial drug dosages from other species to llamas.

The four classes of drugs are as follows, with the chosen representative to be used in this study appearing directly behind.

PENICILLIN	--- AMPICILLIN (Amp-Equine^{R})
SULFA	--- TRIMETHOPRIM (Tribrissen^{R})
FLUOROQUINOLONE	--- ENROFLOXACIN (Baytril^{R})
AMINOGLYCOSIDE	--- TOBRAMYCIN (Nebcin^{R})

Specific Objectives:

The over all objectives of this study were to test the following hypothesis:

The drug disposition characteristics (clearance, half-life, apparent volume of distribution and mean residence time) used to calculate drug dosages and dosing intervals are similar to extrapolated dosages and dosing intervals from other ruminant species for the four commonly used antimicrobials from the four separate classes of drugs.

LITERATURE REVIEW

Both Gram negative and Gram positive bacterial infections are common problems in the llamas (20,21). While vaccination programs have effectively decreased the incidence of clostridial infection, mixed bacterial respiratory, gastrointestinal and reproductive infections are frequently encountered (20). Respiratory and Uterine infections are of particular concern since the damage is frequently extensive prior to the time that overt clinical changes are noted.

Therapy of an infectious disease in the llama is dependent upon the microorganism involved. Numerous penicillins and cephalosporins are available in human medicine. In general they are considered bactericidal agents since they inhibit bacterial cell wall synthesis (22-24). Ampicillin has excellent activity against gram positive organisms and gram negative cocci, but limited activity against gram negative bacilli (22,24). Extended spectrum penicillins, ticarcillin and piperacillin (Ticar, Piperacil etc.), which were the first penicillins to achieve significant activity against gram negative bacteria (24), have structures initially based upon ampicillin.

Penicillins and Cephalosporins are typically handled similarly in the body. They are excreted extensively unmetabolized into the urine via the kidney and have half-lives that are typically under 1.5 hours in humans. Ampicillin's half-lives after intravenous injection in relation to body mass in some species of

mammals and birds are reported as 1.72, 2.0, 1.58, 0.7, 0.53, 0.69, 0.31 and 0.56 hours in buffalo, cow, sheep, goat, pig, rabbit, pigeon and chickens respectively (19).

Trimethoprim, a broad spectrum antibiotic with excellent activity against gram positive organisms is excreted much more slowly ($t_{1/2\beta}$ = 5.5 hours) and metabolized to a greater extent (25). Trimethoprim acts as a folate antagonist by inhibiting dihydrofolate reductase and is often used in combination with the sulfonamide class of drugs (51). Species differences in the rate and extent of the various routes of the elimination processes for trimethoprim reflect the diverse half-life values of the drug; 0.7 to 1.5, 3.2, 4.6 and 10.6 hours in ruminants, horses, dogs and humans respectively (4).

Aminoglycosides like tobramycin have extensive activity against gram negative organisms (24). New antibiotics like the extended activity penicillins and cephalosporins are routinely compared against the aminoglycoside class of drugs for their effectiveness against gram negative organisms (24). In general, tobramycin must be given parenterally. It is excreted essentially unmetabolized into the urine via the kidney and its half-life and clearance is influenced by the renal function of the individual (26-28).

The fluroquinolone class of antibacterials, derivatives of nalidixic acid, have recently received considerable attention in the veterinary field because of their broad spectrum of activity, good absorption after oral administration, low

toxicity, and long elimination half-life (29). They are more active against gram negative organisms, but have so far shown no activity against Pseudomonas aeruginosa (29). Fluroquinolone antibacterials possess a broad spectrum of activity against gram-negative bacteria, such as E.coli, Salmonella, Klebsiella, Proteus, Haemophylus, Pasteurella and Campylobactur (52).

Microbial resistance to fluroquinolone derivatives is slower than to Nalidixic acid, and fluroquinolones extremely low minimum inhibitory concentrations have accelerated their use (29,32-34). Enrofloxacin is similar in structure, activity and use to the human drug ciprofloxacin (29,33). The microbiological activity of the fluroquinolones is thought to be due to the inhibition of bacterial DNA gyrase enzyme (32). This includes cleavage of the DNA backbone, presumably a bactericidal effect. Other mechanisms of activity also may exist. Enrofloxacin has poor oral absorption in ruminant animals and is given parenterally (29). Enrofloxacin's half-life varies considerably between species. Half lives ($t_{1/2\beta}$) of 7.3, 1.4, 1.2, 2.1 and 3.3 hours have been reported for the chicken, turkey, calf, dog and horse respectively (29). Bacterial diseases which have been reported to affect llamas appear to be susceptible to one of the four antimicrobial agents selected in this study.

MATERIALS AND METHODS

Animals: Six sexually mature males in the OSU Camelid research herd were used. All animals were medically sound. Prior to initiation of experiments all animals were held in quarantine for at least one week, vaccinated and dewormed as needed, given a complete physical examination and any routine health care as indicated.

Drug Solutions: Parenteral solutions of ampicillin, trimethoprim, tobramycin, enrofloxacin and ceftiofur were obtained from commercial sources.

Drug solutions were administered by the intravenous bolus route in the sequence of administration shown below. Doses (all doses were equivalent to human dose) given were as follows:

a.	Ampicillin Sodium (Amp-equine^{R})	12 mg/kg
b.	Tobramycin (Nebcin^{R})	1.0 mg/kg
c.	Trimethoprim Sulfa (Tribrissen^{R})	3.0 mg/kg
d.	Enrofloxacin (Baytril^{R})	5.0 mg/kg
e.	Ceftiofur Sodium (Naxcel^{R})	2.2 mg/kg

Blood samples were collected for 12 hours for each treatment followed by three days of no drug administration before the next drug treatment was administered.

From the five drugs only ampicillin, tobramycin, trimethoprim and enrofloxacin are included in this report.

Sampling protocol:

Approximately 24 hours prior to starting the study an indwelling catheter was placed in the jugular vein and fitted with a catheter extension. A local lidocaine block was used to minimize the discomfort associated with catheter placement. Food and water was made available ad libitum. Drug solutions were administered as bolus intravenous dose to the animals through the indwelling catheter, then the catheter was immediately flushed with saline. Blood samples (7 ml Vacutainer) were collected via the catheter immediately before drug administration and 5 min, 10 min, 15 min, 30 min, 45 min, 1 hr, 1.5 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr and 12 hr after drug administration. After a blood sample was collected the catheter was flushed with normal saline solution. After collection of the blood sample, the sample was centrifuged, the plasma decanted and frozen for storage until assay.

Materials used for assays were all analytical grade without further purification. All water was deionized prior to use.

AMPICILLIN ASSAY PROCEDURE

Materials: Ampicillin and β-hydroxyethyl theophylline were obtained from Sigma Chemical Co., Methanol (HPLC grade) was obtained from both Sigma and Aldrich Chemical Co., and Potassium phosphate monobasic was obtained from Aldrich Chemical Co., Inc.

Internal Standard Solution

β-Hydroxyethyl theophylline (25 mg) was dissolved in 100 ml distilled water. Forty ml of this solution was transferred to a 100 ml volumetric flask and distilled water was added to bring up to 100 ml volume. Final concentration was 100 μg/ml.

Sample Preparation

To 0.5 ml of plasma sample, 1.0 ml of methanol was added to deproteinize the plasma. After vortex mixing and centrifuging at 2400x g for 10 min in an Eppendorf centrifuge (model 5415 C), 1.0 ml of supernatant was separated and

25 μl of (100 μg/ml) internal standard (β-hydroxyethyl theophylline) was added and mixed. Then 150 μl of supernatant was injected into the HPLC.

Chromatograph Specifications

The HPLC system (HEWLETT PACKARD, Model LC 1090 M) consisting of a HPLC Pump (Model 100/120 V Ac, 60 Hz, Hewlett Packard) and an Auto sampler (Model 1090 M, #48, Hewlett Packard) was used. The drug was separated on C-18 Column (μBondapak, Water Associate, Inc.) using an UV Detector (Diode Array Detector with Deuterium lamp with a spectrum from 190 nm to 600 nm, UV Absorbance, Hewlett Packard). DAD (Diode Array Detector) signals were set at Sample Wavelength of 205 nm with a Band Width of 4 nm and a Reference Wavelength of 550 nm. The initial threshold was set at 0.1 mAU (absorbance units) with a peak width of 0.1 min (Sampling interval = 640 ms). The flow rate of the mobile phase was 1.0 ml/min and Injection Volume was 150 μl (37) .

Mobile Phase consisted of methanol and phosphate buffer (0.067 M) in the ratio of 35:65 respectively. Mobile phase was filtered through 0.47 μm filter and degassed by sonicating under vacuum for about 15 minutes. The chromatographic condition was isocratic (37).

Standard Curve: Stock solution of ampicillin (25 mg in 100 ml of distilled water) was prepared. Serial dilutions with blank (drug free) llama plasma of this stock solution were made in duplicate to obtain the following concentrations of 1, 5, 10, 25, 50 and 100 μg/ml. Three standard curves (six points each) were run over a period of three weeks. Ampicillin and the internal standard (β-hydroxy ethyl theophylline) peaks were clearly separated and eluted within 15 minutes. Mean retention times for ampicillin and β-hydroxyethyl theophylline were 9.3 and 6.4 minutes respectively.

Care was taken to prepare the stock ampicillin solution (25 mg in 100 ml distilled water) fresh every time the standard curve was run. After samples were prepared they were immediately injected into the HPLC system. The pH of the ampicillin stock solution was between 7.0 to 7.5 to ensure its stability.

Standard curve for ampicillin is shown in Fig 1. Peak height ratios and regression results are shown in Table 5.

TABLE 5. VALUES USED IN THE GENERATION OF THE STANDARD CURVE TO DETERMINE UNKNOWN AMPICILLIN PLASMA CONCENTRATIONS IN LLAMA.

CONCN[1]	DRUG[2]	IS[3]	RESP4	%THEO[5]
1	43.25	435.26	44.02	101.7
5	212.24	425.28	208.90	98.4
10	405.36	436.98	415.00	102.4
25	894.76	426.94	1033.3	115.5
50	1802.27	425.28	2063.8	114.5
100	3560.44	428.50	4124.8	115.8

X^6 =108.05; S.D.[7] =8.03; % CV^8 = 7.68

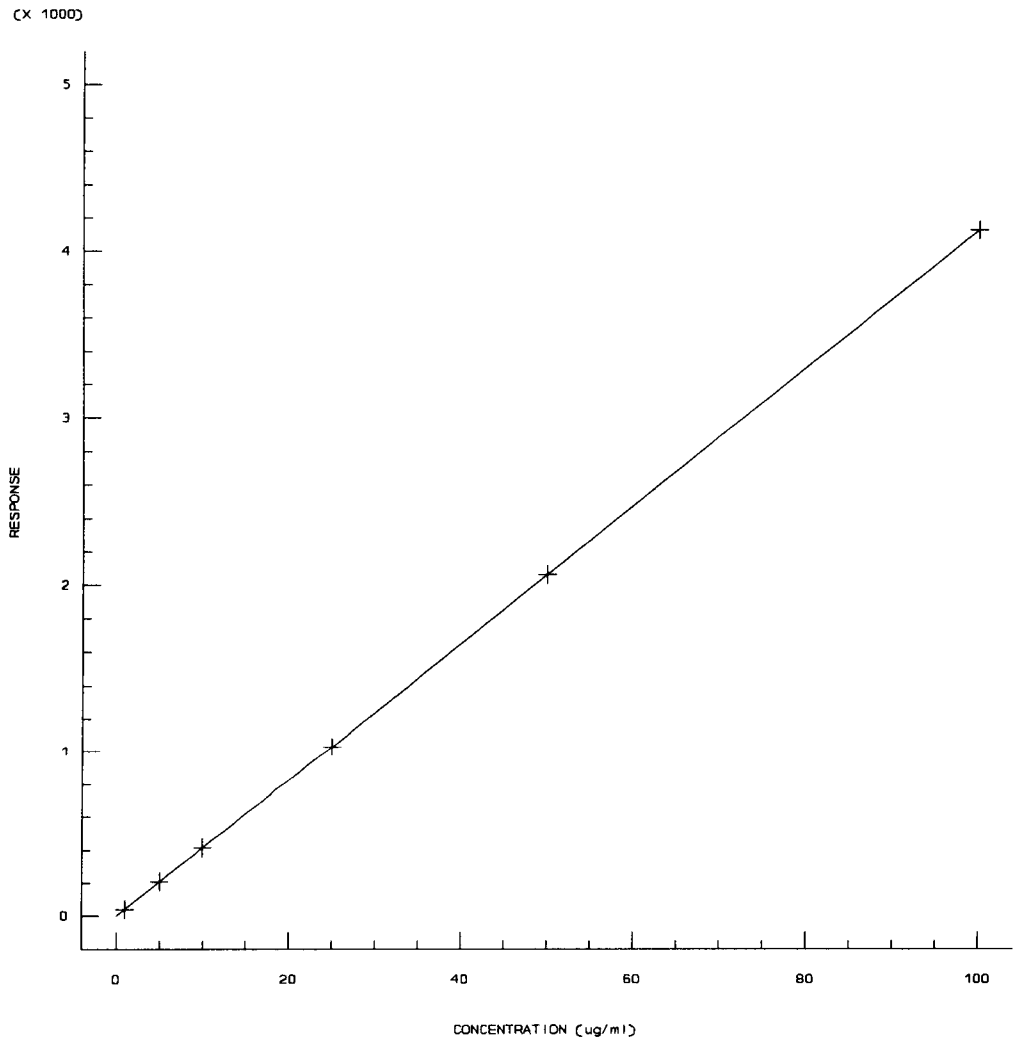
1

---

[1]Results of regression of response on actual concentration:
R^2 = 0.999; Intercept = 2.80; Slope = 41.22

1. Actual concentration of ampicillin (μg/ml)
2. Drug = Area under the peak on chromatogram for ampicillin.
3. IS = Area under the peak on chromatogram for internal standard.
4. Response = (Slope * Amount) + Intercept, which equals predicted ampicillin concentration.
5. %THEO = Percent theoretical concentration of ampicillin.
6. Mean percent theoretical concentration.
7. Standard deviation of the mean.
8. Coefficient of variation as a percent of the mean.

FIG 1. STANDARD CURVE FOR AMPICILLIN FROM HPLC ANALYSIS USED TO DETERMINE UNKNOWN PLASMA AMPICILLIN CONCENTRATIONS.



TOBRAMYCIN ASSAY PROCEDURE

Materials : Tobramycin, gentamycin, tris(hydroxymethyl) aminomethane and 2,4,6-trinitrobenzene-1-sulfonic acid (TNBSA) were obtained from Sigma Chemical Co. Acetonitrile (HPLC grade) and methanol (HPLC grade) were supplied by both the Sigma and Aldrich Chemical Co. Potassium phosphate, monobasic was obtained from Aldrich Chemical Co. C-18 columns, the solid-phase extraction came from J.T. Baker Chemical Co., and the Vac-Elut vacuum chamber was obtained from Analytichem International, Inc.

Reagents and solutions were prepared as follows:

Tris Buffer, 2 mol/L, pH 10.3 was prepared by dissolving 24.2 gm of Tris (hydroxymethyl) aminomethane in distilled water and the volume was adjusted to 100 ml. 2,4,6-Trinitrobenzene-1-sulfonic acid derivatizing solution, 250 g/L was prepared by dissolving 2.5 g of trinitrobenzene sulfonic acid (TNBSA, Sigma) in 10 ml of acetonitrile. Stock Wash Buffer, 1 mol/L was prepared by dissolving 87 gm of potassium phosphate, monobasic in 500 ml of distilled water. Working Wash Solution, a methanol/phosphate buffer solution (0.1 mol/L, pH 8.5, 50/50 V/V) was prepared by transferring 10 ml of stock wash buffer into a 250 ml graduated cylinder and 90 ml of distilled water was added followed with 100 ml of methanol. pH of this solution was adjusted to 8.5 with phosphoric acid. The

mobile phase for the HPLC consisted of 700 ml of acetonitrile and 300 ml of 50 mMol/L phosphate buffer (6.8 gm of potassium phosphate, monobasic per liter of water). pH was adjusted to 3.5 with phosphoric acid. Mobile phase was filtered through 0.47 μm filter and degassed by sonicating under vacuum for 15 minutes.

Internal Standard Solution

Gentamycin (25 mg) was dissolved in 100 ml distilled water. Forty ml of this solution was transferred to 100 ml of volumetric flask and distilled water was added to bring the volume up to 100 ml. Final concentration was 100 μg/ml. Internal standard (Gentamycin) was separately derivatized according to the procedure described below and then added to the derivatized tobramycin sample.

Sample Preparation

Derivatization and Solid-Phase Extraction Procedure

Fifty μl of tobramycin plasma samples were pipetted into 1.5 ml polypropylene tubes and 25 μl of 2 mol/L Tris buffer and 100 μl of acetonitrile were added, vortex mixed and centrifuged for 1 min in an Eppendorf centrifuge (Model 5415 C) at 15,000x g. Supernatant was decanted into a second set of appropriately labeled polypropylene tubes and 30 μl of TNBSA (2,4,6-Trinitrobenzene -

1-Sulfonic acid) was added. Tubes were capped, vortex mixed and placed on a hot plate, heated at 70°c for 30 min. For each sample, a Bond-Elut C-18 extraction column was placed on the top of Vac-Elut vacuum chamber and vacuum was connected to the chamber. Two column volumes of methanol and two column volumes of water was passed through each column. The vacuum was disconnected and each column was filled with 700 μl of working wash solution, followed by approximately 200 μl of derivatized sample. Vacuum was then reconnected to the chamber and three column volumes of working wash solution was passed through each column. Vacuum was disconnected and a rack of labelled glass tubes were placed on the Vac-Elut chamber, corresponding to each Bond-Elut column. Then 300 μl of acetonitrile was pipetted onto each column and vacuum was reconnected. After collecting the eluate in the tubes, 25 μl of similarly derivatized and extracted internal standard (Gentamycin) was added to each tube and mixed. Tobramycin and gentamycin could not be derivatized together as they competed for the derivatizing agent resulting in the incomplete derivatization of the tobramycin. 150 μl of the extracted sample was injected onto the HPLC system (36).

Optimal Conditions for derivatization:

Optimal conditions for derivatization were arrived at by varying reagent concentration, reaction temperature, reaction time and pH. A large

excess of the derivatizing agent (TNBSA) was necessary to yield a single tobramycin derivative quantitatively in < 30 min. (TNBSA reacts with primary amino groups of amino acids and peptides in aqueous solutions at pH 8 without any undesirable side reactions). The resulting trinitrophenyl derivatives had a high molar absorptivity at 340 nm. At temperatures below 70°C there was incomplete derivatization and above 80°C there was substantial decomposition. The optimal conditions for derivatization of tobramycin of 70°c and 30 min reaction time were selected. Below pH 9.0, derivatization was incomplete and slow because of the basic nature of the tobramycin molecule. The optimal pH for this reaction was between 9.5 and 10.0 (36).

Chromatograph Specifications

The HPLC System (Model LC 1090M, HEWLETT PACKARD) consisting of a HPLC Pump (Model 100/120 V Ac, 60 Hz, Hewlett Packard) and an Auto sampler (Model HP 1090 M, # 048, Hewlett Packard) was used. The drug was separated on a C-18 Column (Microsorb -MV, Rainin Instrument Co., Inc.) using a UV detector (Diode Array Detector with a Deuterium lamp with a spectrum of 190 nm to 600 nm, UV Absorbance, Hewlett Packard). DAD(Diode Array Detector) signals were set at Sample Wavelength of 340 nm with a Band width of 4 nm and a Reference Wavelength of 550 nm. The initial threshold was set at 0.1 mAU (absorbance units) with a peak width of 0.1 min

(Sampling interval = 640 ms). The flow rate of the mobile phase was 1.0 ml/min and Injection Volume was 150 μl. The chromatographic condition was isocratic (36).

Standard Curve: Stock solution of tobramycin 25 mg in 100 ml of distilled water was prepared. Serial dilutions with blank (drug free) llama plasma of this stock solution were made in duplicate to obtain the following concentrations of 1, 5, 10, 25, 50 and 100 μg/ml. Standard samples were derivatized and extracted as described for the unknown samples.

Four standard curves (six points each) were run over a period of four weeks. Tobramycin and internal standard (gentamycin) peaks were clearly separated and eluted within 22 minutes. Mean retention time for tobramycin was 19.7 minutes. Internal standard (gentamycin) had three components C_1, C_{1a} and C_2 with retention times for each of the components of 13.8, 16.0 and 17.3 minutes respectively. The component peak with retention time of 13.8 minutes was used as internal standard peak to calibrate tobramycin samples.

Standard curve for Tobramycin is shown in Fig 2. Peak height ratios and regression results are shown in Table 6.

TABLE 6. VALUES USED IN THE GENERATION OF THE STANDARD CURVE TO DETERMINE UNKNOWN TOBRAMYCIN PLASMA CONCENTRATIONS IN LLAMA.

CONCN[1]	DRUG[2]	IS[3]	RESP[4]	%THEO[5]
1	43.234	214.43	41.02	94.8
5	180.01	217.20	177.82	98.8
10	383.06	229.27	348.82	91.1
25	846.50	231.27	861.82	101.7
50	1724.4	219.25	1718.62	99.7
100	3425.2	214.34	3426.82	100.0

X^6 = 97.65; S.D.[7] = 3.86; % CV^8 = 3.95

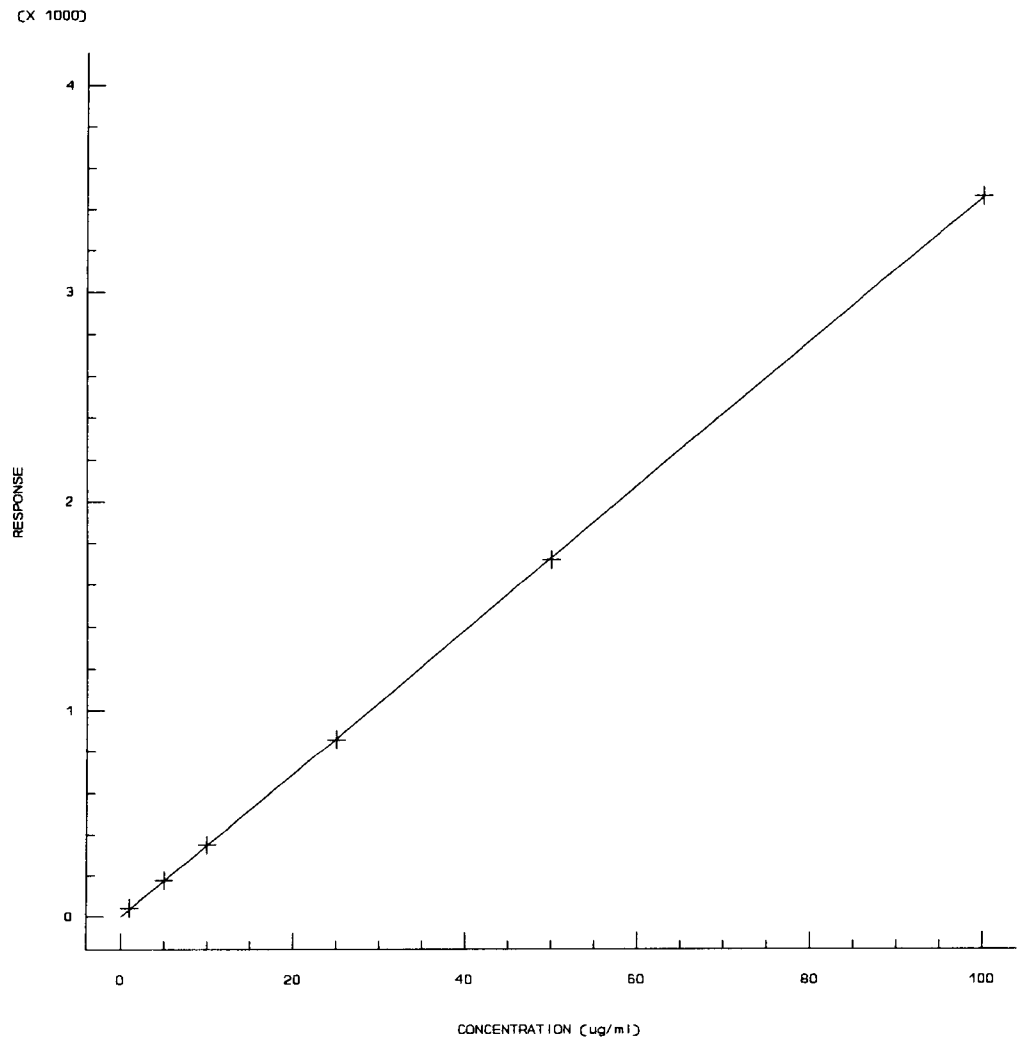
[2]

---

[2] Results of regression of response on actual concentration: R^2 = 1.000; Intercept = 6.82; Slope = 34.2

1. Actual concentration of tobramycin (μg/ml)
2. Drug = Area under the peak concentration for tobramycin.
3. IS = Area under the peak on chromatogram for gentamycin the internal standard.
4. Response = (Slope * Amount) + Intercept, which equals the predicted tobramycin concentration.
5. %THEO = Percent theoretical concentration of tobramycin.
6. Mean percent theoretical concentration.
7. Standard deviation of the mean.
8. Coefficient of variation as a percent of the mean.

FIG 2. STANDARD CURVE FOR TOBRAMYCIN FROM HPLC ANALYSIS USED TO DETERMINE UNKNOWN PLASMA TOBRAMYCIN CONCENTRATIONS.



TRIMETHOPRIM ASSAY PROCEDURE

Materials: Trimethoprim and antipyrine were obtained from Sigma Chemical Co. Potassium phosphate, dibasic and perchloric acid were supplied by Aldrich Chemical Co., Inc. Methanol (HPLC grade) was obtained from both the Sigma and Aldrich Chemical Co.

Internal Standard Solution

Antipyrine (100 mg) was dissolved in 100 ml distilled water. 10 ml of this solution was transferred to a 100 ml volumetric flask and distilled water was added to bring the volume up to 100 ml. Final concentration was 100 μg/ml.

Sample Preparation

To 100 μl of the trimethoprim plasma sample, 100 μl of 1 mol/L perchloric acid and 25 μl of 100 μg/ml of internal standard (antipyrine) were added together. The mixture was vortexed and centrifuged for 5 minutes in an Eppendorf centrifuge (model 5415 C) at 5000x g. To 150 ml of the supernatant, 150 μl of (0.5 mol/L) dibasic potassium phosphate was added and after through mixing the sample was centrifuged again for 5 minutes at 5000x g. The 30 μl of the final supernatant was injected onto the HPLC.

Chromatograph Specification

The HPLC system consisting of a HPLC Pump (Model M-600 A; Water Associate, Inc) and a WISP auto sampler (Model 710 B Water Associate, Inc) was used. The drug was separated on a μBondapak C-18 Column (Water Associate, Inc) using a UV detector (Series 440, UV Absorbance; Water Associate, Inc) with mercury lamp and filter at 280 nm and sensitivity set at 0.05 absorbance units. A recorder (Linear Inc.) set at a chart speed 6 cm/hr received signal from the detector at a 10 mV scale. The mobile phase consisted of methanol and phosphate buffer (0.05 mol/L, pH 4.4) in the ratio of 30:70 respectively. Mobile phase was filtered through 0.47 μm filter and degassed by sonicating under vacuum for about 15 minutes. The flow rate of the mobile phase was 1 ml/min and the injection volume was 30 μl. The chromatographic condition was isocratic (25).

Standard Curve: A stock solution of trimethoprim (15 mg in 100 ml of distilled water) was prepared. Serial dilutions with blank (drug free) llama plasma with this stock solution were made in duplicate to obtain the following trimethoprim concentrations of 1, 2, 5, 10, 15 and 20 μg/ml.

Four standard curves (six points each) were run over a period of three weeks. Trimethoprim and the internal standard (antipyrine) peaks were clearly separated and eluted within 10 minutes.

Mean retention times for trimethoprim and antipyrine were 6.0 and 7.9 minutes respectively.

Standard curve for trimethoprim is shown in Fig 3. Peak height ratios and regression results are shown in Table 7.

TABLE 7. VALUES USED IN THE GENERATION OF THE STANDARD CURVE TO DETERMINE UNKNOWN TRIMETHOPRIM PLASMA CONCENTRATIONS IN LLAMA.

CONCN[1]	DRUG[2]	IS[3]	PHR[4]	INV[5]	%THEO[6]
1	1.05	8.9	0.118	0.877	87.7
2	2.35	8.93	0.264	2.087	104.3
5	5.45	8.8	0.620	5.037	100.7
10	11.03	9.1	1.212	9.943	99.4
15	15.33	8.3	1.845	15.188	101.2
20	21.58	8.95	2.412	19.887	99.4

7,5

X^{7} = 98.78

S.D.[8] = 5.22

% CV [9] = 5.28

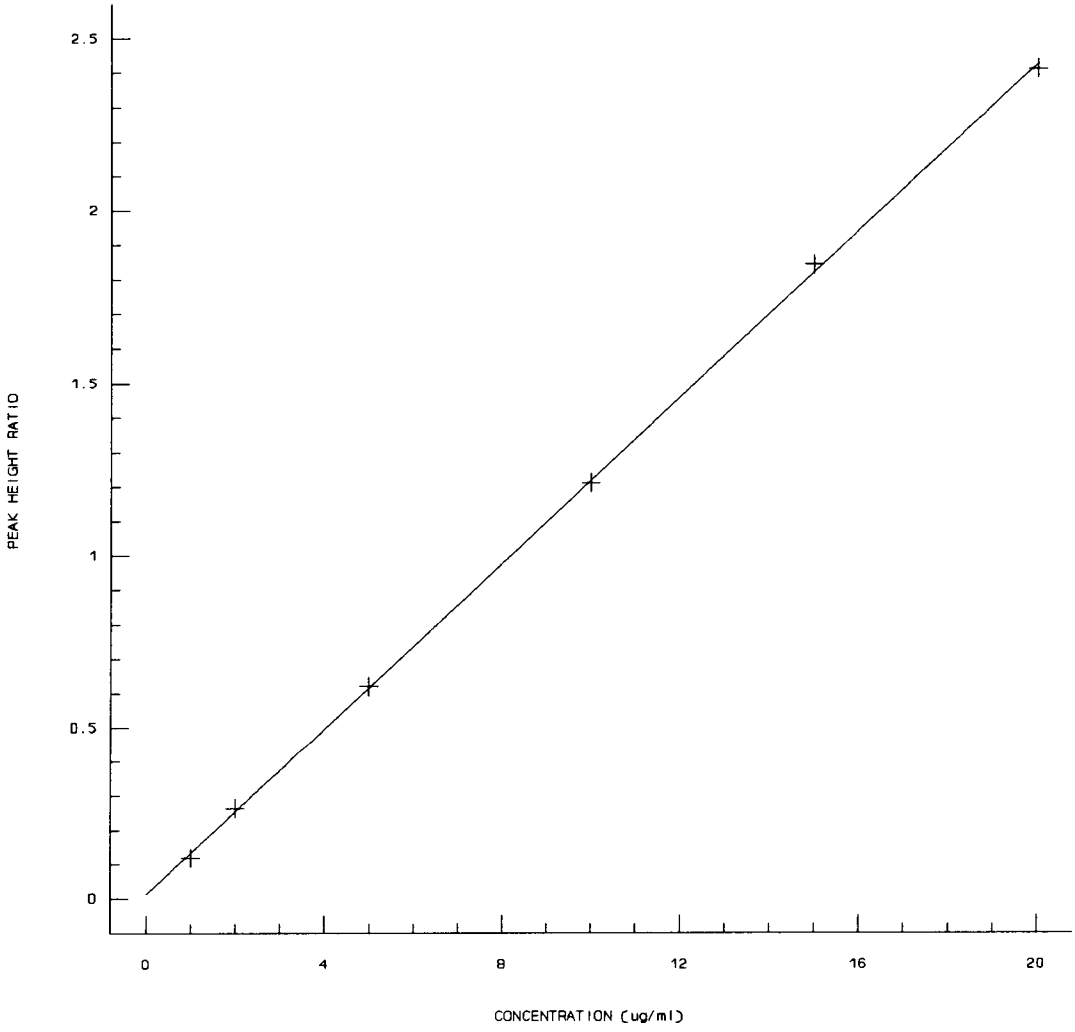
3

---

[3]Results of regression of Peak Height Ratio on actual concentration: R = 0.9998; Intercept = 0.01222; Slope = 0.1206

1. Actual concentration of trimethoprim (μg/ml)
2. Drug = Height of drug peak on chromatogram (mm) for trimethoprim.
3. IS = Height of internal standard peak on chromatogram (mm) for antipyrine.
4. PHR = Peak Height Ratio (Drug/IS: Trimethoprim/Antipyrine).
5. Inverse = Inversely estimated trimethoprim concentrations calculated from PHR and regression parameters by the equation: Inverse = (PHR - Intercept)/Slope
6. % THEO = Percent Theoretical Concentration of trimethoprim.
7. Mean percent theoretical concentration.
8. Standard deviation of the mean.
9. Coefficient of variation as a percent of the mean.

FIG 3. STANDARD CURVE FOR TRIMETHOPRIM FROM HPLC ANALYSIS USED TO DETERMINE UNKNOWN PLASMA TRIMETHOPRIM CONCENTRATIONS.



ENROFLOXACIN ASSAY PROCEDURE

Materials: Enrofloxacin was obtained from Mobay Corporation. Cefazolin was obtained from Sigma Chemical Co. Methanol (HPLC grade) and acetonitrile (HPLC grade) were supplied by both the Sigma and Aldrich Chemical Co., Inc. Potassium phosphate, monobasic was obtained from Aldrich Chemical Co.

Internal Standard Solution

Cefazolin (100 mg) was dissolved in 100 ml distilled water. Ten ml of this solution was transferred to 100 ml volumetric flask and distilled water was added to bring up to 100 ml volume. Final concentration was 100 μg/ml.

Sample Preparation

To 50 μl of enrofloxacin plasma sample, 50 μl of acetonitrile was added and the mixture was vortex mixed and centrifuged (10,000x g) in an Eppendorf centrifuge (model 5415 C). Twenty five μl of (100 μg/ml) internal standard (cefazolin) was added to the deproteinized plasma sample and mixed. Twenty μl of the clear supernatant was injected onto the HPLC.

Chromatograph Specification

The HPLC system consisting of a HPLC Pump (model M-600 A; Water Associates, Inc) and a WISP auto sampler (model 710 B; Water Associates, Inc) was used. The drug was separated on a μBondapak C-18 Column (Water Associates, Inc) using a UV detector (Series 440, UV Absorbance; Water Associates, Inc) with a mercury lamp and filter at 280 nm and a sensitivity set at 0.05 absorbance units. A recorder (Linear Inc.) set at chart speed of 6 cm/hr received signal from the detector at a 10 mV scale. The mobile phase consisted of methanol and phosphate buffer (67 mMol/L, pH 3.5) in the ratio of 35:65 respectively. Mobile phase was filtered through 0.47 μm filter and degassed by sonicating under vacuum for about 15 minutes. The flow rate of the mobile phase was 1.0 ml/min and the injection volume was 20 μl. The chromatographic condition was isocratic (35).

Standard Curve: A stock solution of enrofloxacin (25 mg in 100 ml distilled water) was prepared. Serial dilutions with blank (drug free) llama plasma of this stock solution were made in duplicate to obtain the following enrofloxacin concentrations of 1, 2, 4, 8, 16, 24 and 32 μg/ml.

Six standard curves (seven points each) were run over a period of four weeks. Enrofloxacin and the internal standard (cefazolin) were clearly separated and eluted within 10 minutes with the mean retention times of 8.4 and 5.2

minutes respectively. Internal standard (cefazolin) was freshly prepared each time the standard curve was run.

Standard curve for enrofloxacin is shown in Fig 4. Peak height ratios and regression results are shown in Table 8.

TABLE 8. VALUES USED IN THE GENERATION OF THE STANDARD CURVE TO DETERMINE UNKNOWN ENROFLOXACIN PLASMA CONCENTRATIONS IN LLAMA.

CONCN[1]	DRUG[2]	IS[3]	PHR[4]	INV[5]	%THEO[6]
1.00	0.3	6.7	0.045	1.39	139.7
2.00	0.6	7.6	0.080	2.13	106.4
4.01	1.1	7.1	0.155	3.69	92.1
8.02	3.15	9.05	0.348	7.72	96.3
16.04	6.75	9.15	0.738	15.87	98.93
24.12	10.3	8.95	1.151	24.49	101.5
32.08	14.5	9.6	1.509	31.97	99.7

X^{7} = 104.9; S.D.[8] = 14.76; % CV^{9} = 13.87

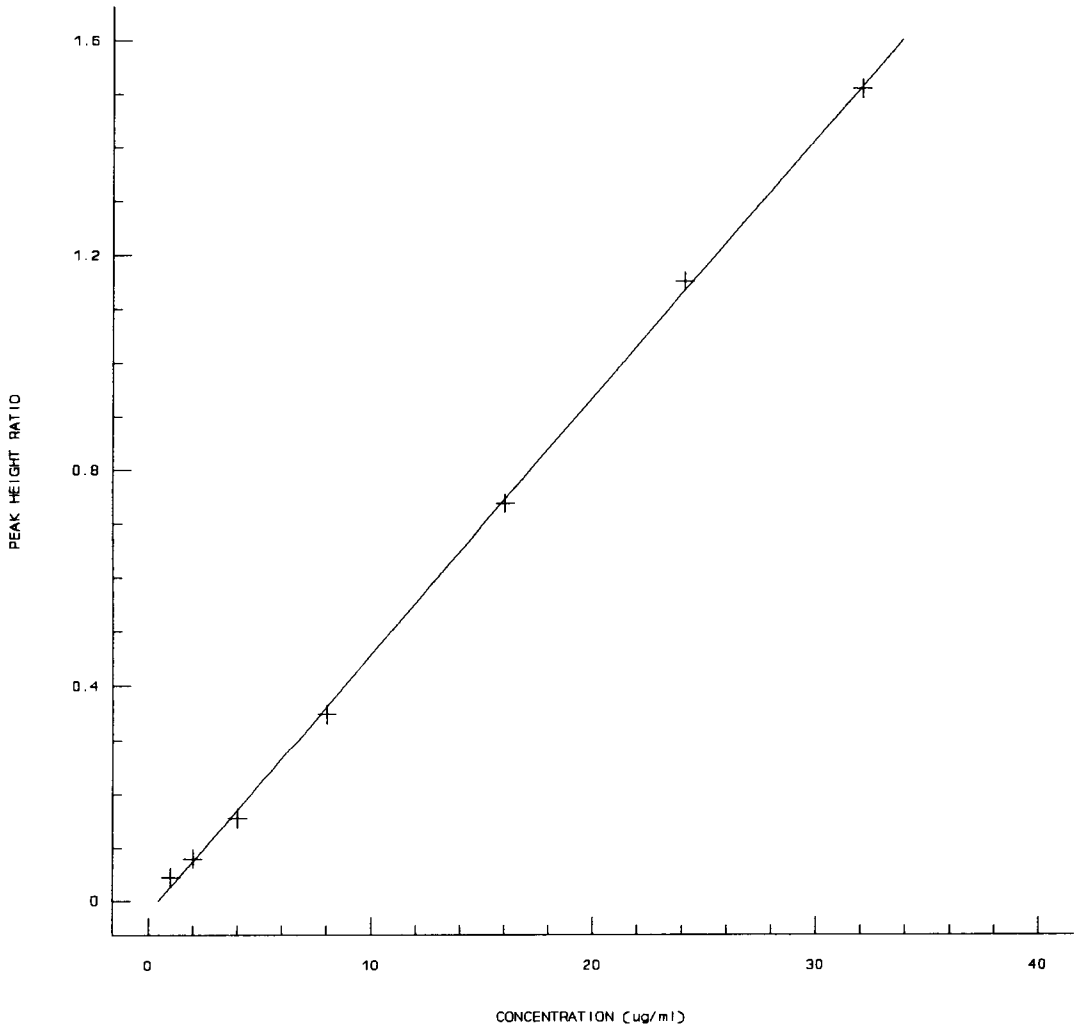
4

---

[4] Results of regression on actual concentration: R = 0.999; Intercept = - 0.02195; Slope = 0.04789

1. Actual concentration of enrofloxacin (μg/ml).
2. Drug = Height of drug peak on chromatogram (mm) for enrofloxacin.
3. IS = Height of internal standard peak on chromatogram (mm) for cefazolin.
4. PHR = Peak Height Ratio (Drug/IS: Enrofloxacin/Cefazolin).
5. Inverse = Inversely estimated enrofloxacin concentration calculated from PHR and regression parameters by the equation: Inverse = (PHR - Intercept)/Slope.
6. % THEO = Percent of theoretical concentration for enrofloxacin.
7. Mean percent of the mean.
8. Standard deviation of the mean.
9. Coefficient of variation as a percent of the mean.

FIG 4. STANDARD CURVE FOR ENROFLOXACIN FROM HPLC ANALYSIS USED TO DETERMINE UNKNOWN PLASMA ENROFLOXACIN CONCENTRATIONS.



Pharmacokinetic Analysis:

Ampicillin, Tobramycin, Trimethoprim and Enrofloxacin plasma concentrations versus time data were analyzed using RSTRIP Computer Software (30) to fit the data. Using both the compartmental and non-compartmental approaches the pharmacokinetic parameters of half-lives, clearances, apparent volumes of distribution and mean residence times were calculated (31,38,39,40).

The following specific objectives were addressed or elucidated:

1. To determine and compare the disposition and elimination characteristics of the representative member of the four most commonly used classes of antimicrobials after bolus IV administration.
2. Characterize the pharmacokinetic parameters of the antimicrobials (clearance, half-life, apparent volume of distribution and mean residence time) to identify if significant differences in these pharmacokinetic parameters occur than would be expected by extrapolation or scaling from other species.
3. Estimate the therapeutic duration of action and dosing intervals necessary for the adequate therapy for each representative agent of the therapeutic classes.

The plasma concentration time data of each antimicrobial was fit using RSTRIP Computer Software. The data was weighted $1/(concn)^2$ to get a

best fit. Drug concentrations in llama plasma after bolus IV administration were individually fitted to a 1, 2 or 3 compartment open model for kinetic analysis, with the first order elimination from the central compartment. Non-linear least squares fit of each set of data was obtained using the number of terms required for each llama.

Goodness of fit was based on improvement in sum of squares, model selection criterion and values of coefficient of determination.

RESULTS

AMPICILLIN

Mean plasma concentrations of ampicillin at each sampling time are shown in table 9. The post-distribution clearance values for ampicillin are presented in table 10. The clearance values indicate ampicillin follows linear pharmacokinetics in llama.

Plasma concentrations of ampicillin as a function of time after bolus IV administration in each llama were fitted to a two exponential equation. Non-linear least squares fit of each set of data to the general equation:

$$C_p = \sum A_i e^{-\lambda i t} \qquad \text{Eq. 1.}$$

was obtained but using the number of terms required for each subject. Where C_p is the ampicillin plasma concentration, λ_i are the exponents, A_i are the pre-exponential coefficients and t is time.

Plasma ampicillin concentrations after IV bolus administration for all six llama's required two exponential function:

$$Cp = A_1 e^{-\lambda 1 t} + A_2 e^{-\lambda 2 t} \qquad \text{Eq. 2.}$$

to characterize the declining plasma ampicillin concentrations as a function of time. C_p is the ampicillin plasma concentration, λ_1 and λ_2 are distribution and elimination rate constants and t is time.

The determination of the best fit compartment model and estimates of the model dependent pharmacokinetic parameters (41-44) of A_1, A_2, λ_1, λ_2, K_{el} and V_c were made by the use of RSTRIP (30). Statistical moments theory was used to compute the non-compartmental model pharmacokinetic parameters of mean residence time (MRT), apparent volume of distribution (V_{darea}), apparent volume of distribution at steady-state (V_{dss}), body clearance (CL_B) and area under the curve (AUC) (42-45).

Plasma ampicillin concentration vs. time curves from all six llama's were analyzed individually. Mean values for each pharmacokinetic parameter for a compartmental and a non-compartmental model were computed with their standard deviation and are shown in tables 11 and 12 respectively.

Mean curve of plasma concentrations vs. time after IV bolus administration of ampicillin is shown in fig 5.

Pharmacokinetics of ampicillin after bolus IV administration in all llama's fitted (correlation coefficient > 0.99) a two compartmental model with $\alpha = 1.12 \pm 0.58$ and $\beta = 0.208 \pm 0.050$. The plasma half-life ($t_{1/2\beta}$) of ampicillin was 3.50 ± 1.01 hours.

The mean residence time (MRT) of ampicillin was 5.01 $\pm$ 0.66 hours and volume of distribution at steady state (V_{dss}) was 0.277 $\pm$ 0.085 l/kg.

TABLE 9. DRUG CONCENTRATION Vs. TIME DATA OF SIX LLAMA'S RECEIVING AN IV DOSE OF 12 mg/kg OF AMPICILLIN.

TIME	CONCN (μg/ml) LLAMA #1	CONCN LLAMA #2	CONCN LLAMA #3	CONCN LLAMA #4	CONCN LLAMA #5	CONCN LLAMA #6	MEAN OF CONCN	SD OF CONCN
0 MIN	-----	-----	-----	-----	-----	-----	----	----
5 MIN	56.73	61.22	48.87	46.45	40.47	59.88	52.27	8.28
10 MIN	55.95	61.69	44.54	42.07	41.21	60.60	51.01	9.46
15 MIN	50.74	58.26	40.21	39.58	38.32	56.31	47.24	8.98
30 MIN	48.13	56.23	35.98	35.55	33.95	54.50	44.06	10.13
45 MIN	44.18	53.32	37.84	31.28	30.46	49.25	41.05	9.47
1 HR	42.20	50.71	31.29	26.43	26.14	44.67	36.91	10.35
1.5 HR	35.91	46.46	28.48	24.36	25.02	41.41	33.61	9.12
2 HR	32.18	41.08	22.20	19.89	20.95	37.23	28.92	9.13
3 HR	27.67	33.94	18.37	15.78	17.78	31.00	24.09	8.28
4 HR	20.94	29.34	14.93	11.23	13.62	25.75	19.30	7.22
6 HR	14.92	23.18	11.05	8.42	9.65	18.73	14.32	5.75
8 HR	7.49	14.01	7.84	6.10	5.50	12.65	8.93	3.53
12 HR	3.11	5.62	3.90	3.26	2.03	6.80	4.12	1.76

TABLE 10. CLEARANCE IN l/kg Vs. TIME IN SIX LLAMA'S RECEIVING AN IV DOSE OF 12 mg/kg AMPICILLIN CALCULATED AFTER THE DISTRIBUTION PHASE HAS ENDED.

TIME	CLEARANCE (lit/hr/kg)						MEAN OF CLEA-RANCE	SD OF CLEA-RANCE
	LLAMA #1	LLAMA #2	LLAMA #3	LLAMA #4	LLAMA #5	LLAMA #6		
3 HR	0.033	0.019	0.065	0.093	0.046	0.041	0.050	0.026
4 HR	0.061	0.015	0.070	0.060	0.075	0.041	0.054	0.021
6 HR	0.037	0.012	0.052	0.058	0.048	0.035	0.040	0.016
8 HR	0.074	0.025	0.058	0.065	0.078	0.043	0.057	0.019
12 HR	0.046	0.022	0.058	0.062	0.065	0.034	0.048	0.017

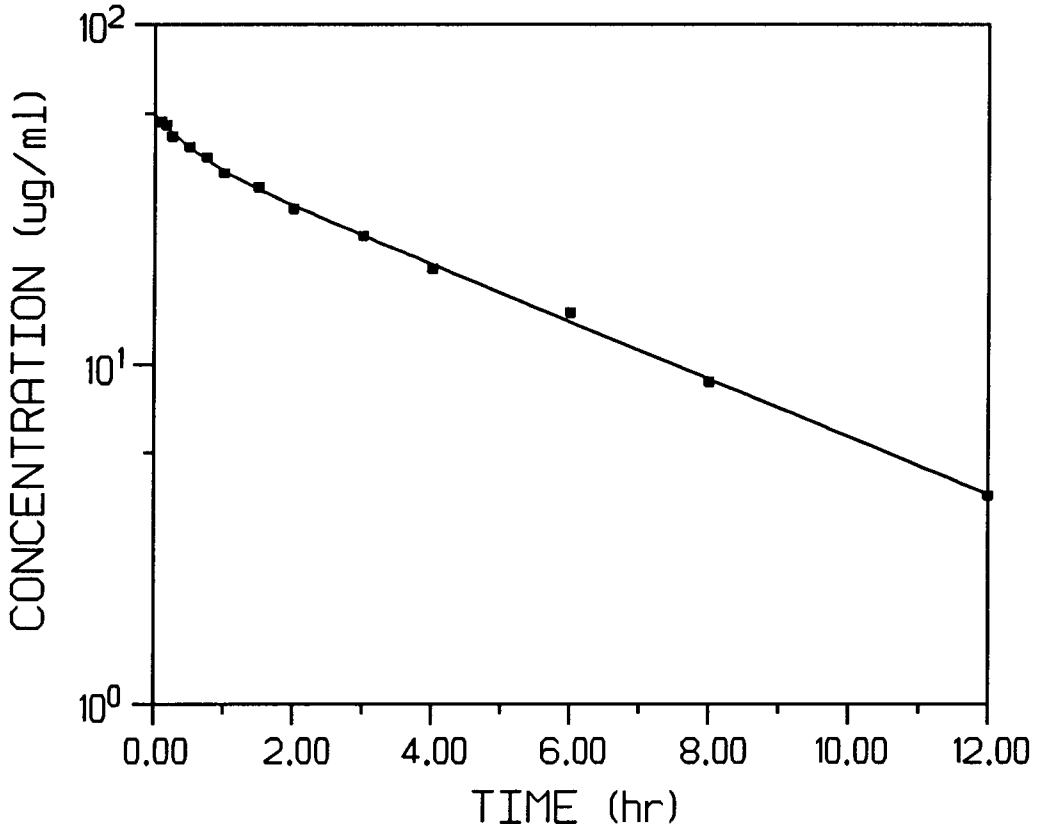
TABLE 11. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY COMPARTMENTAL MODEL METHODS FOR AMPICILLIN IN SIX LLAMA'S AFTER IV INJECTION OF 12 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
β	h^{-1}	0.236	0.261	0.166	0.155	0.261	0.168	0.208	0.050
α	h^{-1}	6.206	0.482	1.024	0.894	0.867	1.250	1.12	0.58
A_1	μg/ml	53.31	24.30	29.05	20.84	34.80	50.42	35.45	13.58
A_2	μg/ml	8.34	15.42	19.13	25.44	10.09	12.30	15.12	6.35
$t_{1/2\alpha}$	h	0.112	1.44	0.676	0.775	0.799	0.555	0.726	0.380
$t_{1/2\beta}$	h	2.94	2.65	4.17	4.48	2.65	4.12	3.50	0.50
V_c	l/kg	0.195	0.302	0.249	0.259	0.273	0.191	0.245	0.044
r		0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.000

TABLE 12. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY NON-COMPARTMENTAL MODEL METHODS FOR AMPICILLIN IN SIX LLAMA'S AFTER IV INJECTION OF 12 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
MRT	h	4.21	4.90	5.53	5.53	4.11	5.78	5.01	0.66
CL_B	l/h/kg	0.053	0.038	0.062	0.074	0.078	0.039	0.057	0.017
V_{darea}	l/kg	0.224	0.143	0.373	0.475	0.300	0.230	0.291	0.119
AUC_{0-12}	μg.h/ml	213.81	295.47	169.71	142.14	145.14	269.72	206.00	63.80
$AUC_{0-\infty}$	μg.h/ml	227.07	321.71	193.51	163.24	152.89	309.52	228.00	72.74
Vd_{ss}	l/kg	0.222	0.183	0.343	0.407	0.282	0.224	0.277	0.085

FIG 5. MEAN PLASMA CONCENTRATION Vs. TIME PROFILE IN SIX LLAMA'S GIVEN AN IV DOSE OF 12 mg/kg OF AMPICILLIN INCLUDING A BEST FIT LINE FIT BY RSTRIP.



TOBRAMYCIN

Mean plasma concentrations of tobramycin at each sampling time are shown in table 13. The post-distribution clearance values for tobramycin are presented in table 14. The clearance values indicate tobramycin follows linear pharmacokinetics in llama.

Tobramycin plasma concentrations following IV bolus administration for all six llama's required a two exponential function to characterize the declining plasma tobramycin concentrations.

The determination of best fit compartmental model and estimates of the model dependent pharmacokinetic parameters were made as described for ampicillin. Similarly the non-compartmental model pharmacokinetic parameters were determined using statistical moments theory. Plasma tobramycin concentrations vs. time curves from all six llama's were analyzed individually.

Mean values for each pharmacokinetic parameter for a compartmental and a non-compartmental model were computed with their standard deviation and are shown in tables 15 and 16 respectively. Mean curve of plasma concentrations after bolus IV administration of the tobramycin is shown in fig 6.

Pharmacokinetics of tobramycin after IV bolus administration in all six llama's fitted (correlation coefficient > 0.99) a two compartmental model

with α = 1.91 $\pm$ 0.894 and β = 0.162 $\pm$ 0.037. The plasma half-life ($t_{1/2\beta}$) of tobramycin was 4.51 $\pm$ 1.26 hours. The mean residence time (MRT) of tobramycin was 5.53 $\pm$ 1.79 hours and volume of distribution at steady state (Vdss) was 0.138 $\pm$ 0.049 l/kg.

TABLE 13. DRUG CONCENTRATION Vs. TIME DATA OF SIX LLAMA'S RECEIVING IV DOSE OF 1 mg/kg OF TOBRAMYCIN.

TIME	CONCN (μg/ml) Llama #1	CONCN Llama #2	CONCN Llama #3	CONCN Llama #4	CONCN Llama #5	Llama #6	MEAN OF CONCN	SD OF CONCN
0 MIN	----	----	----	----	----	----	----	----
5 MIN	18.25	19.01	15.5	15.91	13.47	11.17	15.55	2.92
10 MIN	18.26	13.31	13.05	13.48	11.48	9.10	13.11	3.01
15 MIN	16.12	9.57	11.44	11.71	9.30	8.38	11.09	2.77
30 MIN	12.18	7.48	8.86	9.99	7.45	6.44	8.73	2.09
45 MIN	11.03	5.64	8.95	9.02	6.61	7.49	8.12	1.92
1 HR	8.34	4.63	7.25	6.79	6.72	4.69	6.40	1.46
1.5 HR	7.82	3.66	6.38	5.61	4.30	4.12	5.31	1.58
2 HR	5.65	4.06	4.74	5.27	3.47	3.23	4.40	0.97
3 HR	4.28	2.9	3.62	4.53	2.69	3.03	3.51	0.66
4 HR	2.63	NA	3.71	3.43	3.07	2.06	3.00	0.58
6 HR	1.93	2.18	2.76	2.39	1.81	1.49	2.09	2.37
8 HR	0.99	1.78	1.24	1.38	1.11	0.93	1.24	0.30
12 HR	0.63	1.40	0.72	0.69	0.79	0.38	0.77	0.33

TABLE 14. CLEARANCE IN l/kg Vs. TIME IN SIX LLAMA'S RECEIVING AN IV DOSE OF 1 mg/kg OF TOBRAMYCIN CALCULATED AFTER THE DISTRIBUTION PHASE HAS ENDED.

TIME	CLEARANCE (lit/hr/kg)						MEAN OF CLEA-RANCE	SD OF CLEA-RANCE
	Llama #1	Llama #2	Llama #3	Llama #4	Llama #5	Llama #6		
3 HR	0.026	0.064	0.016	0.039	0.041	0.009	0.033	0.019
4 HR	0.046	NA	0.029	0.015	0.021	0.056	0.033	0.017
6 HR	0.015	0.030	0.019	0.052	0.041	0.024	0.029	0.014
8 HR	0.031	0.019	0.028	0.025	0.038	0.034	0.031	0.008
12 HR	0.011	0.012	0.018	0.020	0.014	0.012	0.015	0.003

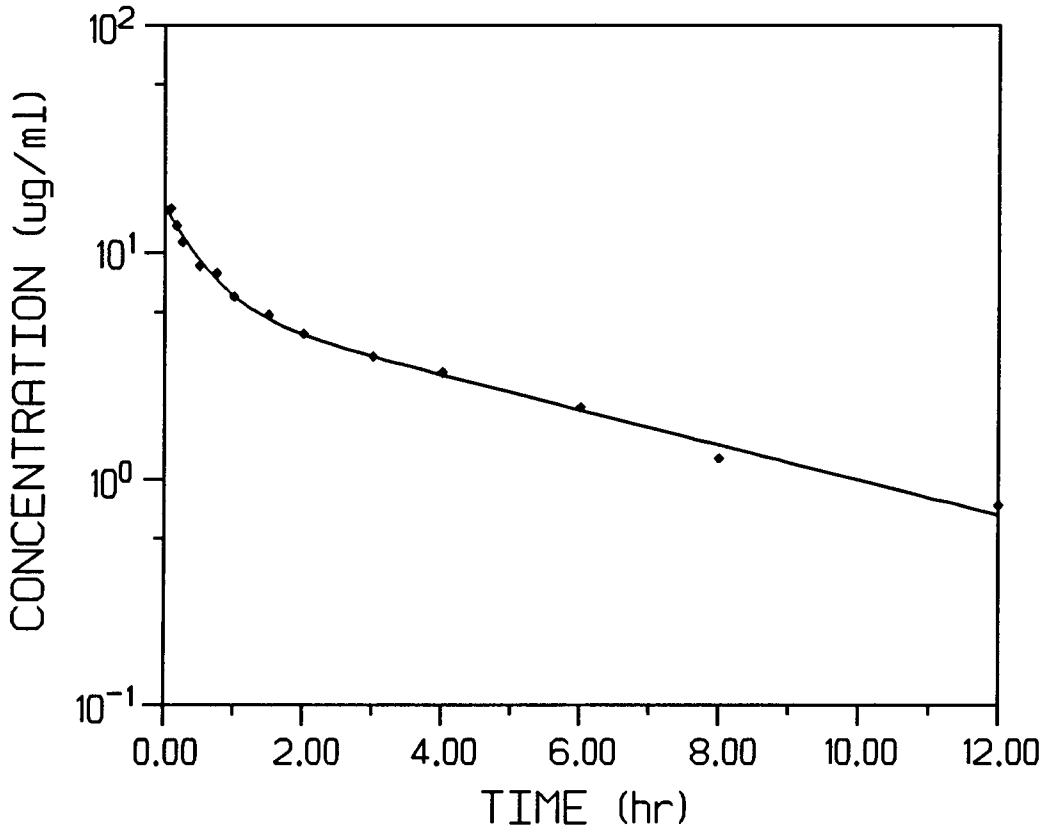
TABLE 15. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY COMPARTMENTAL MODEL METHODS FOR TOBRAMYCIN IN SIX LLAMA'S AFTER IV INJECTION OF 1 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
β	h^{-1}	0.166	0.101	0.198	0.205	0.152	0.151	0.162	0.037
α	h^{-1}	0.842	3.302	1.980	2.50	1.60	1.22	1.91	0.894
A_1	μg/ml	4.31	4.29	7.25	7.81	4.41	3.66	5.29	1.76
A_2	μg/ml	14.02	15.96	8.51	9.39	8.91	6.97	10.63	3.52
$t_{1/2\alpha}$	h	0.823	0.210	0.349	0.280	0.434	0.567	0.443	0.223
$t_{1/2\beta}$	h	4.16	6.86	3.49	3.37	4.56	4.59	4.51	1.26
V_c	l/kg	0.054	0.049	0.060	0.058	0.075	0.094	0.065	0.016
r		0.999	0.998	0.999	0.999	0.997	0.997	0.998	0.001

TABLE 16. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY NON-COMPARTMENTAL MODEL METHODS FOR TOBRAMYCIN IN SIX LLAMA'S AFTER IV INJECTION OF 1 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
MRT	h	4.12	8.91	4.56	4.47	5.61	5.51	5.53	1.79
CL_B	l/h/kg	0.024	0.021	0.024	0.024	0.029	0.033	0.026	0.004
V_{darea}	l/kg	0.141	0.210	0.124	0.117	0.190	0.221	0.167	0.045
AUC_{0-12}	μg.h/ml	39.03	34.65	37.40	38.53	29.91	25.98	34.25	5.26
$AUC_{0-\infty}$	μg.h/ml	42.55	47.26	40.76	41.76	34.58	29.93	39.47	6.57
V_{dss}	l/kg	0.097	0.189	0.112	0.084	0.162	0.184	0.138	0.049

FIG 6. MEAN PLASMA CONCENTRATION Vs. TIME PROFILE IN SIX LLAMA'S GIVEN AN IV DOSE OF 1 mg/kg OF TOBRAMYCIN INCLUDING A BEST FIT LINE FIT BY RSTRIP.



TRIMETHOPRIM

Mean plasma concentrations of trimethoprim at each sampling time are shown in table 17. The post distribution clearance values for trimethoprim are presented in table 18. The clearance values of trimethoprim indicate it follows linear pharmacokinetics in llama.

Trimethoprim plasma concentrations following IV bolus administration in all six llama's were best described by a bi-exponential equation. The determination of best fit compartmental model and estimates of the model dependent pharmacokinetic parameters were made as described for ampicillin. Similarly the non-compartmental pharmacokinetic parameters were determined using statistical moment theory. Plasma trimethoprim concentrations vs. time curves from all six llama's were analyzed individually.

Mean values for each pharmacokinetic parameters for a compartmental and a non-compartmental model were computed with their standard deviation and are shown in tables 19 and 20 respectively. Mean trimethoprim plasma concentrations vs. time after IV bolus administration is shown in fig 7.

Pharmacokinetics of trimethoprim after bolus IV administration in all the llama's fitted (correlation coefficient > 0.99) a two compartment model with $\alpha = 2.86 \pm 2.27$ and $\beta = 0.151 \pm 0.079$.

The plasma half-life ($t_{1/2\beta}$) of trimethoprim was 4.29 $\pm$ 2.52 hours. The mean residence time (MRT) of trimethoprim was 4.83 $\pm$ 1.74 hours and volume of distribution at steady state (Vdss) was 0.404 $\pm$ 0.151 l/kg.

TABLE 17. DRUG CONCENTRATION Vs. TIME DATA IN SIX LLAMA'S RECEIVING AN IV DOSE OF 3 mg/kg OF TRIMETHOPRIM.

TIME	CONCN (μg/ml) Llama #1	CONCN Llama #2	CONCN Llama #3	CONCN Llama #4	CONCN Llama #5	CONCN Llama #6	MEAN OF CONCN	SD OF CONCN
0 MIN	----	----	----	----	----	----	----	----
5 MIN	15.99	14.29	14.95	15.35	16.41	8.98	14.33	2.71
10 MIN	14.86	9.21	14.34	9.79	14.17	7.11	11.58	3.27
15 MIN	11.58	10.45	12.39	10.83	12.1	6.37	10.62	2.20
30 MIN	10.42	8.6	10.2	9.61	9.75	5.28	8.98	1.91
45 MIN	10.46	7.78	9.49	8.00	9.28	4.78	8.3	1.98
1 HR	9.1	6.29	9.32	3.38	8.22	3.62	6.65	2.66
1.5 HR	7.91	5.85	6.86	5.46	7.17	3.29	6.09	1.63
2 HR	7.73	5.08	6.16	4.24	6.2	2.31	5.28	1.87
3 HR	6.05	4.05	6.10	3.16	5.05	1.98	4.4	1.64
4 HR	4.57	2.8	4.73	2.36	4.51	1.21	3.36	1.44
6 HR	2.91	1.46	2.88	1.31	2.65	0.68	1.98	0.97
8 HR	1.81	1.09	1.99	0.61	1.98	0.75	1.37	0.59
12 HR	0.97	0.65	1.05	0.34	1.17	0.26	0.74	0.39

TABLE 18. CLEARANCE IN l/kg Vs. TIME IN SIX LLAMA'S RECEIVING AN IV DOSE OF 3 mg/kg OF TRIMETHOPRIM CALCULATED AFTER THE DISTRIBUTION PHASE HAS ENDED.

TIME	CLEARANCE (lit/hr/kg)						MEAN OF CLEA-RANCE	SD OF CLEA-RANCE
	Llama #1	Llama #2	Llama #3	Llama #4	Llama #5	Llama #6		
3 HR	0.062	0.129	0.116	0.003	0.062	0.291	0.111	0.098
4 HR	0.071	0.210	0.115	0.072	0.035	0.338	0.140	0.113
6 HR	0.053	0.181	0.114	0.069	0.079	0.030	0.088	0.053
8 HR	0.059	0.084	0.145	0.052	0.044	0.293	0.113	0.095
12 HR	0.039	0.072	0.057	0.044	0.040	NA(*)	0.050	0.012

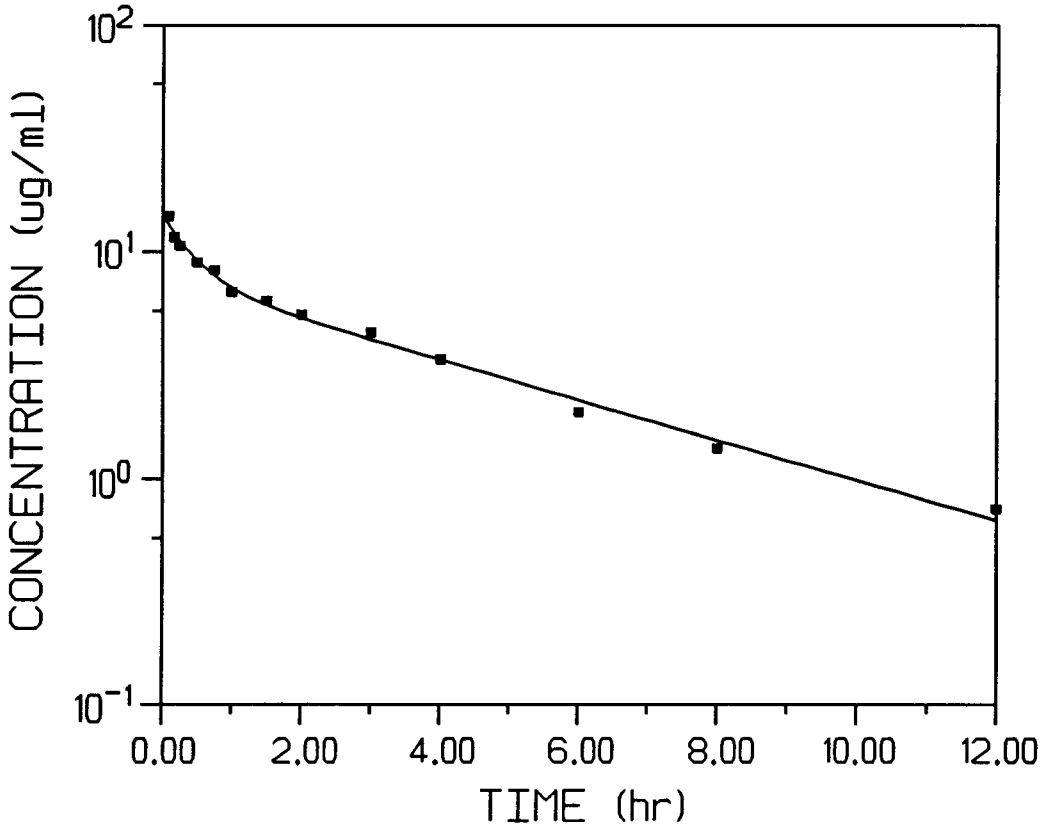
TABLE 19. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY COMPARTMENTAL MODEL METHODS FOR TRIMETHOPRIM IN SIX LLAMA'S AFTER IV INJECTION OF 3 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
β	h^{-1}	0.216	0.074	0.256	0.187	0.176	0.246	0.151	0.079
α	h^{-1}	6.97	0.45	3.53	2.46	2.55	1.22	2.86	2.27
A_1	μg/ml	11.25	1.48	5.96	9.44	8.60	2.02	6.46	4.03
A_2	μg/ml	9.36	9.27	10.77	6.97	8.71	6.72	8.63	1.54
$t_{1/2\alpha}$	h	0.099	1.53	0.196	0.281	0.271	0.568	0.49	0.532
$t_{1/2\beta}$	h	3.21	9.35	2.71	3.69	3.95	2.82	4.29	2.52
V_c	l/kg	0.146	0.279	0.179	0.183	0.173	0.343	0.217	0.075
r		0.999	0.998	0.998	0.999	0.999	0.998	0.998	0.001

TABLE 20. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY NON-COMPARTMENTAL MODEL METHODS FOR TRIMETHOPRIM AFTER IV INJECTION OF 3 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
MRT	h	4.51	7.79	3.49	5.07	5.35	2.76	4.83	1.74
CL_B	l/h/kg	0.056	0.074	0.114	0.056	0.057	0.218	0.096	0.063
V_{darea}	l/kg	0.260	0.980	0.445	0.301	0.326	0.880	0.532	0.315
AUC_{0-12}	μg.h/ml	49.51	32.22	25.27	47.91	46.45	12.57	35.66	14.89
$AUC_{0-\infty}$	μg.h/ml	53.41	40.55	26.35	53.23	52.40	13.72	39.94	16.63
Vd_{ss}	l/kg	0.254	0.576	0.398	0.286	0.306	0.604	0.404	0.151

FIG 7. MEAN PLASMA CONCENTRATION Vs. TIME PROFILE IN SIX LLAMA'S GIVEN AN IV DOSE 3 mg/kg OF TRIMETHOPRIM INCLUDING A BEST FIT LINE FIT BY RSTRIP.



ENROFLOXACIN

Mean plasma concentration of enrofloxacin at each sampling time are shown in table 21. The post distribution clearance values for enrofloxacin are presented in table 22. The clearance values of enrofloxacin indicate it follows linear pharmacokinetics in llama.

Enrofloxacin plasma concentrations following bolus IV administration for all six llama's required a two exponential function to characterize the declining enrofloxacin plasma concentrations.

The determination of best fit compartmental model and estimation of the model dependent pharmacokinetic parameters were made as described for ampicillin. Similarly the non-compartmental model pharmacokinetic parameters were determined using statistical moment theory. Plasma enrofloxacin concentrations vs. time curves from all six llama's were analyzed individually.

Mean values for each pharmacokinetic parameters for a compartmental and a non-compartmental model were computed with their standard deviation and are shown in tables 23 and 24 respectively. Mean enrofloxacin plasma concentrations vs. time curve after IV bolus administration is shown in fig 8.

Pharmacokinetics of enrofloxacin after bolus IV administration fitted correctly in all llama's (correlation coefficient > 0.99) to a two compartment

model with α = 2.48 $\pm$ 1.21 and β = 0.205 $\pm$ 0.069. The plasma half-life ($t_{1/2\beta}$) enrofloxacin was 3.94 $\pm$ 2.13 hours. The mean residence time (MRT) of enrofloxacin was 4.95 $\pm$ 2.87 hours and volume of distribution at steady state was 0.346 $\pm$ 0.098 l/kg.

TABLE 21. DRUG CONCENTRATION Vs. TIME DATA OF SIX LLAMA'S RECEIVING AN IV DOSE OF 5 mg/kg OF ENROFLOXACIN.

TIME	CONCN (μg/ml) Llama #1	CONCN Llama #2	CONCN Llama #3	CONCN Llama #4	CONCN Llama #5	CONCN Llama #6	MEAN OF CONCN	SD OF CONCN
0 MIN	----	----	----	----	----	----	----	----
5 MIN	25.00	30.5	35.1	25.35	34.67	24.95	29.26	4.43
10 MIN	22.39	17.86	29.46	24.96	27.89	22.17	24.12	4.22
15 MIN	17.6	15.6	24.87	22.55	27.53	18.36	21.08	3.63
30 MIN	15.18	11.72	20.59	18.59	18.25	14.86	16.53	3.28
45 MIN	12.26	10.69	17.43	15.91	15.78	12.26	14.05	2.66
1 HR	9.16	8.62	13.02	12.46	15.55	11.82	11.77	2.56
1.5 HR	9.11	8.02	11.33	9.14	12.91	8.08	9.76	1.97
2 HR	8.63	7.42	8.8	7.85	10.07	7.06	8.30	1.09
3 HR	4.46	6.47	7.00	4.37	8.7	3.95	5.82	1.87
4 HR	3.65	6.09	5.86	4.06	7.15	3.61	5.07	1.48
6 HR	3.05	4.79	3.89	3.2	5.8	2.68	3.90	1.18
8 HR	1.23	NA	1.05	1.3	3.48	1.31	1.67	1.34
12 HR	0.56	3.27	0.65	0.59	1.55	0.94	1.26	1.05

TABLE 22. CLEARANCE IN l/kg Vs. TIME IN SIX LLAMA'S RECEIVING AN IV DOSE OF 5 mg/kg OF ENROFLOXACIN CALCULATED AFTER THE DISTRIBUTION PHASE HAS ENDED.

TIME	CLEARANCE (lit/hr/kg)						MEAN OF CLEA-RANCE	SD OF CLEA-RANCE
	Llama #1	Llama #2	Llama #3	Llama #4	Llama #5	Llama #6		
3 HR	0.219	0.069	0.173	0.053	0.039	0.241	0.132	0.098
4 HR	0.069	0.031	0.023	0.041	0.052	0.038	0.042	0.016
6 HR	0.031	0.061	0.036	0.047	0.028	0.063	0.044	0.015
8 HR	0.146	NA	0.128	0.132	0.066	0.146	0.124	0.029
12 HR	0.054	0.044	0.057	0.027	0.051	0.035	0.045	0.012

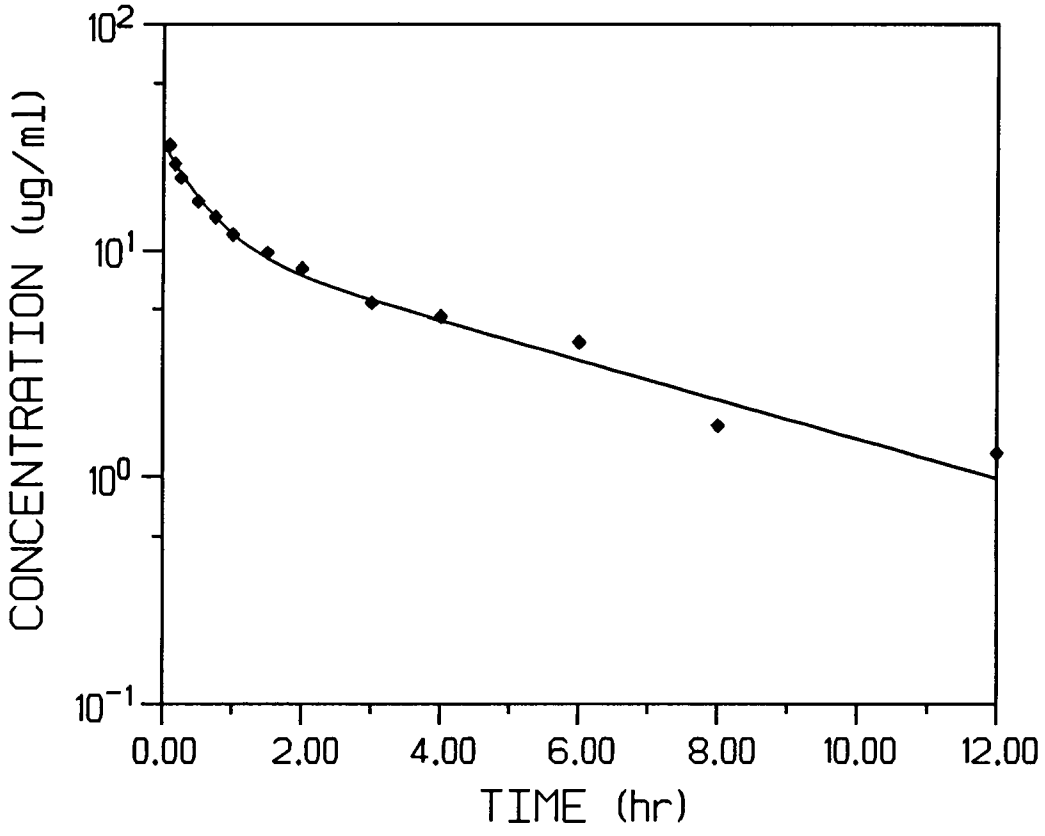
TABLE 23. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY COMPARTMENTAL MODEL METHODS FOR ENROFLOXACIN IN SIX LLAMA'S AFTER IV INJECTION OF 5 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
β	h^{-1}	0.254	0.089	0.232	0.305	0.192	0.161	0.205	0.069
α	h^{-1}	2.23	4.31	1.32	2.74	3.22	1.07	2.48	1.21
A_1	μg/ml	10.86	8.92	9.45	16.86	16.02	5.76	11.31	4.31
A_2	μg/ml	16.02	23.28	19.17	21.62	23.60	18.24	20.32	3.01
$t_{1/2\alpha}$	h	0.310	0.161	0.525	0.253	0.215	0.647	0.352	0.191
$t_{1/2\beta}$	h	2.73	7.76	2.98	2.27	3.61	4.32	3.94	2.13
V_c	l/kg	0.186	0.155	0.175	0.130	0.126	0.208	0.163	0.032
r		0.999	0.995	0.999	0.999	0.999	0.999	0.998	0.001

TABLE 24. PHARMACOKINETIC PARAMETER VALUES OBTAINED BY NON-COMPARTMENTAL MODEL METHODS FOR ENROFLOXACIN AFTER IV INJECTION OF 5 mg/kg.

Para-meter	Units	Llama No. 1	2	3	4	5	6	Mean	SD
MRT	h	3.44	10.63	3.37	2.91	4.81	4.52	4.95	2.87
CL_B	l/h/kg	0.100	0.048	0.091	0.079	0.055	0.094	0.078	0.021
V_{darca}	l/kg	0.394	0.534	0.389	0.259	0.287	0.588	0.408	0.130
AUC_{0-12}	μg.h/ml	47.92	71.06	52.65	61.73	82.45	47.69	60.58	13.98
$AUC_{0-\infty}$	μg.h/ml	49.96	105.24	55.14	63.16	90.78	52.91	69.53	22.95
Vd_{ss}	l/kg	0.344	0.505	0.305	0.231	0.265	0.427	0.346	0.098

FIG 8. MEAN PLASMA CONCENTRATION Vs. TIME PROFILE IN SIX LLAMA'S GIVEN AN IV DOSE OF 5 mg/kg OF ENROFLOXACIN INCLUDING A BEST FIT LINE BY RSTRIP.



DISCUSSION AND CONCLUSION

AMPICILLIN

The elimination half-life ($t_{1/2\beta}$) of ampicillin in llamas was 3.50 $\pm$ 0.50 hours, where as half-lives of 1.09, 0.79, 0.96 and 1.6 hours have been reported for human, sheep, mice and horses respectively (11-14). The mean residence time (MRT) in llamas was 5.01 $\pm$ 0.66 hours, where as mean residence times of 0.40 and 0.36 hours have been reported for human and sheep respectively (11,14). The volume of distribution at steady state (V_{dss}) in llamas was 0.277 $\pm$ 0.085 l/kg, where as 0.269, 0.156, 0.377 and 2.5 l/kg has been reported for human, sheep, mice and horses respectively (11-14). Ampicillin is eliminated more slowly from llamas than other ruminants.

The dose and dosing interval of ampicillin in sheep is 10 mg/kg/day and 40 mg/kg/day in horses (11,12). In humans ampicillin dosing regime is 250 mg every 6 hours orally for a 70 kg person. Ampicillin given 12 mg/kg of body weight every 12 hours administered parenterally to llamas provides ampicillin concentrations effective against ampicillin sensitive bacteria. From this dosing regime a C_{max} (peak) and C_{min} (trough) plasma concentrations of 47 $\pm$ 15 μg/ml and 3.9 $\pm$ 1.3 μg/ml would be obtained at steady state.

A reduction in ampicillin dosing to 12 mg/kg/day or 6 mg/kg every 12 hours appears to be appropriate for most microbial infections in llamas.

TOBRAMYCIN

The elimination half-life ($t_{1/2\beta}$) tobramycin in llamas was 4.51 $\pm$ 1.26 hours, where as half-lives of 1.84 and 1.59 hours have been reported for cats and humans respectively (15,16). The mean residence time (MRT) in llamas was 5.53 $\pm$ 1.79 hours, where as mean residence time of 1.75 hours has been reported in cats (15). The volume of distribution at steady state (V_{dss}) in llamas was 0.138 $\pm$ 0.049 l/kg, compared to the volume of distribution at steady state of 0.10 and 0.241 l/kg has been reported for cats and humans respectively (15,16).

When determining tobramycin dosage regimen, the frequency of administration of tobramycin depends on the effective blood concentration desired. Minimal toxic concentrations of tobramycin have not been determined in the llama. In people, it is recommended that peak plasma concentration be <12 μg/ml (46). Dosage recommendations calculated to induce an average steady-state plasma concentration (C^{ss} ave) that is within the therapeutic range (4 to 8 μg/ml), for many severe infections (46). Pharmacokinetic values determined after IV administration of tobramycin in llamas were used for this calculation.

The dose and dosing interval of tobramycin in cats is 2 mg/kg every 8 hours (15). The human dose and dosing interval of 1 mg of tobramycin/kg of body weight given IV, every 8 hour, will yield a C^{ss} ave of approximately 5.0 μg/ml which is within the therapeutic range. The peak (C_{max}) plasma tobramycin concentration in llama will be approximately 10.0 $\pm$ 4.0 μg/ml and the trough (C_{min}) plasma concentration will be 2.7 $\pm$ 1.1 μg/ml following a dosing regimen of 1 mg/kg of body weight every 8 hours administered parenterally. This trough level in humans may cause nephrotoxicity and would need to be reduced to half this value by reducing the dose in half. Adequate therapy could still be obtained for most bacteria with peak level of 5 μg/ml and a trough of about 2 μg/ml. Nephrotoxicity in llamas has not been reported as yet to be a concern after aminoglycoside administration and may not occur.

TRIMETHOPRIM

The elimination half-life ($t_{1/2\beta}$) of trimethoprim in llamas was 4.29 $\pm$ 2.52 hours, where as half-lives of 1.65 and 3.92 and 14.6 hours have been reported for rat, horse and man respectively (17,18,49). The mean residence time (MRT) in llamas was 4.83 $\pm$ 1.74 hours, compared to the mean residence time of 0.86 hours has been reported in rat (17). The volume of distribution at steady

state (V_{dss}) in llamas was 0.404 $\pm$ 0.151 l/kg, where as volume of distribution at steady state of 2.03 l/kg and 1.78 l/kg has been reported in rat and man (17,49).

On the basis of pharmacokinetic profile observed, trimethoprim 3 mg/kg of body weight every 12 hours administered parenterally should provide trimethoprim concentrations effective against trimethoprim sensitive bacteria. From this dosing regimen a C_{max} (peak) and C_{min} (trough) plasma concentrations of 8.8 $\pm$ 3.8 μg/ml and 1.5 $\pm$ 0.6 μg/ml respectively would be obtained at steady state. This dosing schedule is efficacious in attaining therapeutic levels against the most common pathogens reported earlier (47). The minimum inhibitory concentrations for trimethoprim against E. coli, Klebriella, Enterobacter and Proteus range from 1.0 μg/ml to 4.0 μg/ml (50).

The dose and dosing interval of trimethoprim in horse is 4 mg/kg every day in horses (18). In man, trimethoprim dose and dosing interval is 3 mg/kg every 6 hours (49).

ENROFLOXACIN

The elimination half-life ($t_{1/2\beta}$) of enrofloxacin in llamas was 3.94 $\pm$ 2.13 hours, where as half-lives of 4.1, 18.7, 2.7, 2.5 and 24.4 hours have been reported for turkey, chicken, calf, rabbit and fish respectively (7-10). The mean residence time (MRT) in llamas was 4.95 $\pm$ 2.87 hours, where as mean residence

time of 30.2 hours has been reported in fish (10). The volume of distribution at steady state (V_{dss}) in llamas was 0.346 ± 0.098 l/kg, where as volume of distribution at steady state of 0.63, 0.93 and 2.77 l/kg has been reported for calf, rabbit and fish respectively (8-10).

Enrofloxacin has a broad spectrum of antimicrobial activity, with minimal inhibitory concentration (MIC) values ranging from 0.008 to 0.75 μg/ml for >100 various bacterial pathogens and enrofloxacin achieved concentrations higher than the MIC in several tissues (48). Llamas which are a species that frequently have infections caused by organisms susceptible to enrofloxacin, the plasma concentrations of enrofloxacin after IV bolus administration were higher than the mean MIC values needed for many organisms. Moreover maximal plasma concentrations remained high over several hours.

Enrofloxacin administered parenterally at 5 mg/kg of body weight every 12 hours should provide enrofloxacin concentrations effective against most bacterial pathogens. From this dosing regimen a C_{max} (peak) and C_{min} (trough) plasma concentrations of 15 ± 4.8 μg/ml and 1.4 ± 0.4 μg/ml can be obtained at steady state.

The dose and dosing interval of enrofloxacin in calf and rabbits is 5 mg/kg every 12 hours (8,9), compared to fish it is 5 mg/kg/day (10). In dogs enrofloxacin dosing regimen of 2.5 mg/kg every 12 hours or 5 mg/kg/day orally has been reported (48).

CONCLUSION

Doses given in this study provided adequate anti-microbial drug concentrations in llamas. Dose and dosing regimens outlined are adequate but not always extrapolated directly from other ruminant animals. In some instances the dose and dosing interval matched human dosing more closely than ruminant animals but in other instances this was not the case. Anti-microbial dosing in llamas still needs further study to elucidate better therapeutic regimens.

BIBLIOGRAPHY

1. Butt MT, Weldon A, Step D De, Lahunta A, Huxtable CR. Encephalitic Listeriosis in two adult llamas: Clinical Presentations, Lesions and Immunofluorescence of Listeria Monocytogenes in Brainstem Lesions. Cornell Vet. (1991) 81:25-58.

2. Fowler ME. Medicine and Surgery of South American Camelids. Iowa State Press. (1989): 6.

3. Johnson LW. Veterinary Clinics of North America. W.B. Saunders Company, Philadelphia 19105. (1989) 5:1, 37-54

4. Baggot JD. Clinical Pharmacokinetics in Veterinary Medicine: Clinical Pharmacokinetics. (1992) 22(4): 254-273.

5. Prescott JF, Baggot JD. Antimicrobial therapy in Veterinary Medicine. Blackwell Scientific Publication. Boston/Oxford (1988).

6. Thedford TR, Johnson LW. Infectious Diseases of New World Camelids: Llama Medicine. (1987) 145-157.

7. Conzelman GM, McMilan RA, Baggot JD. Pharmacokinetics of Enrofloxacin in turkeys: Proc West Pharmacol Soc. (1986) 29: 321-323.

8. Davidson JN, Conzelman GM, Baggot JD. Pharmacokinetics of Enrofloxacin in Preruminant and ruminant calves: Proc West Pharmacol Soc. (1986) 29: 129-132.

9. Broome RL, Brooks DL, Babish JG, Copeland DD and Conzelman GM. Pharmacokinetic properties of enrofloxacin in rabbits: Am J Vet Res. (1991) 52(11): 1835-1841.

10. Browser PR, Wooster GA, Stleger J, Babish JG. Pharmacokinetics of Enrofloxacin in fingerling rainbow trout: J Vet Pharmacol Therap. (1992) 15: 62-71.

11. Oukessou M, Toutain PL. Effect of water disposition on absorption (oral, intramuscular) and disposition of Ampicillin in sheep: J Vet Pharmacol Therap. (1992) 15: 421-432.

12. Chang-Xio L, Jia-Rong W, Yi-Li L. Pharmacokinetics of Sulbactam and Ampicillin in mice and in dogs: Antimicrob Agents Chemother. (1991) 12: 44-56.

13. Durr A. Comparison of the Pharmacokinetics of Penicillin G and Ampicillin in the horse: Research in Veterinary Science. (1976) 20: 24-29.

14. Ripa S, Fuigi F, Manuela P. Pharmacokinetics of Sulbactam/Ampicillin in humans after intravenous and intramuscular injection: Chemotherapy. (1990) 36: 185-192.

15. Antoinette DJ, Hatch RC, Wilson RC. Pharmacokinetics of Tobramycin in cats: Am J Vet Res. May (1988) 49(5): 608-612.

16. Simon VK, Mosinger EV and Malerczy V. Pharmacokinetic studies of tobramycin and gentamycin. Antimicrobial Agents and Chemotherapy. Apr (1973) 3,4: 445-450

17. Yu-Hsing Tu, Loyd VA Jr, Vincent MF, Donald DA. Pharmacokinetics profile of sulphamethoxine-trimethoprim in rat. Journal of Pharmaceutical Sciences. July (1989) 78(7): 556-560.

18. Carli S, Sonzogni O, Villa R. Pharmacokinetic profile of Sulfamethoxine-trimethoprim in horses after intravenous, intramuscular and oral administration: Research in Veterinary Science. (1993) 54: 184-188.

19. Lashev LD, Pashov DA. Interspecies variations in plasma half-life of ampicillin, amoxycillin, sulphadimidine and sulphacetamide related to variations in body mass. Research in Veterinary Science (1992) 53: 160-164.

20. Thedford TR, Johnson LW. Infectious Diseases of New World Camelids: Llama Medicine. (1987) 145-157.

21. Garry F. Clinical Pathology of Llamas: Llama Medicine. (1989) 55-70.

22. Donowitz GR, Mandell GL. Beta-Lactam Antibiotics Part 1: New Eng J Med. (1988) 318(7): 419-426.

23. Donowitz GR, Mandell GL. Beta-Lactam Antibiotics Part 2: New Eng J Med. (1988) 318(8): 490-500.

24. Wright AJ, Wilkowsky CJ. The Penicillins: Mayo Clinic Proceed. (1991) 66: 1047-1063.

25. Hutabarat RM, Unadkat JD, Sahajwalla C, McNamara S, Ramey B and Smith AL. Disposition of Drugs in Cystic Fibrosis I: Sulphamethoxazole and Trimethoprim: Clin Pharmacol Ther. (1991) 49: 402-409.

26. Mann HJ, Fuhs DW, Rahmat A et.al. Altered Aminoglycoside Pharmacokinetics in critically ill patients with sepsis: Clin Pharmacy. (1987) 6: 148-153.

27. Fang G, Brennen C, Wagener M, Swanson D, Hill M, Zadecky L, DeVine J, Yu VL. Use of ciprofloxacin vs. Use of Aminoglycosides for therapy of complicated urinary tract infection: Prospective, Randomized, Clinical and Pharmacokinetic Study: Antimicrob Agents and Chemother. (1991) 35: 1849-1855.

28. Aronoff GR, Brier RA, Sloan RS, Brier ME. Interactions of Ceftazidime and Tobramycin in patients with normal and impaired renal function. Antimicrob agents and Chemother. (1990) 34: 1139-1142.

29. Vancutsen PM, Babish JG, Schwart WS. The Fluroquinolone antimicrobials: Structure, Antimicrobial activity, Pharmacokinetics, Clinical use in domestic animals and toxicity: Cornell Vet. (1990) 8: 173-186.

30. RSTRIP, Ver 1.0. MicroMath Scientific Software, Salt Lake City, Utah 84121.

31. Riegalman S, Collier P. Application of statistical moment theory to the evaluation of in-vivo dissolution time and absorption time: Journal of Pharmacokinetics and Biopharmaceutics. (1980) 8: 509-534.

32. Walker RC, Wright AJ. The Fluoroquinolones. Mayo Clin. Proc.(1991) 66: 1249-1259.

33. Prescott JF, Yielding KM. In Vitro Susceptibility of selected Veterinary Bacteria to Ciprofloxacin, Enrofloxacin and Norfloxacin: Can J Vet Res. (1990) 54: 195-197.

34. Nix DE, De Vito JM. Ciprofloxacin and Norfloxacin. Two fluoroquinolone Antimicrobials: Clin Pharm. (1987) 6: 105-117.

35. Weber A, Chaffin D, Smith A and Ophein KE. Quantitation of ciprofloxacin in Body fluids by HPLC: Antimicrob Agents and Chemother. apr (1985): 531-534.

36. Kabra PM, Bhatnager K, Nelson MA, Wall JH, Marton LJ. Liquid-Chromatographic Determination of Tobramycin in Serum with Spectrophotometric Detection: Clin Chem. (1983) 29(4): 672-674.

37. Marzo A, Monti N and Ripmonti M. High-performance liquid chromatographic assay of ampicillin and its prodrug lenampicillin: Journal of Chromatography. (1990) 507: 235-239.

38. Yamaoka K, Nakagawa T, Uno T. Statistical Moments in Pharmacokinetics: Journal of Pharmacokinetics and Biopharmaceutics. (1978) 6: 547-557.

39. Wagner JG. Linear Pharmacokinetic Equations Allowing Direct Calculation of Many Needed Pharmacokinetic Parameters from the coefficients of Many Needed Polyexponential Equations which have been fitted to the Data: Journal of Pharmacokinetics and Biopharmaceutics. (1976) 4: 443-467.

40. Adrian D, Pascal K. Estimation of Noncompartmental Parameters: A Technical Note: Journal of Pharmacokinetics and Biopharmaceutics. (1989) 17(1): 131-137.

41. Baggot JD. Principles of drug disposition in domestic animals: The basis of veterinary clinical pharmacology, Philadelphia: WB Saunders Co. (1977)

42. Gibaldi M, Peerier D. Pharmacokinetics. 2nd ed. New York: Marcel Dekker Inc. (1988)

43. Rowland M, Tozer TN. Clinical Pharmacokinetics: Concepts and applications. 2nd ed. Philadelphia: Lea and Febiger. (1989)

44. Wagner JG. Dose regimen calculations. Fundamentals of clinical pharmacokinetics. Hamilton III: Drug Intelligence Publications Inc. (1979)

45. Dunne A, King P. Estimation of non-compartmental parameters: A technical note. J Pharmacokinet Biopharm (1989) 17: 131-137.

46. Sande MA, Mandel GL. Antimicrobial agents: The aminoglycosides. In: Gilman AG, Goodman LS, Gilman A. Goodman and Gilman's the pharmacological basis of therapeutics. New York, Mac Millan (1980): 1162-1180.

47. Bushby SRM. Combined antibacterial action in vitro of trimethoprim and sulfonamides. Postgraduate Medical Journal (1969) 45: 10-18.

48. Walker RD, Stein GE, Mauptman JG, MacDonald KH. Pharmacokinetic evaluation of enrofloxacin administered orally to healthy dogs. Am J Vet Res (1992) 53,12: 2315-2319.

49. Stevens RC, Laizure SC, Sanders PL and Stein DS. Multiple Dose Pharmacokinetics of 12 Milligrams of Trimethoprim and 60 Milligrams of Sulfamethoxazole per kilogram of body weight per day in healthy volunteers. Antimicrobial Agents and Chemotherapy, Mar (1993) 37,3: 448-452.

50. Rylance GW, George RH, Healing DE and Roberts DGV. Single dose pharmacokinetics of trimethoprim. Archives of Disease in Childhood (1985) 60: 29-33.

51. Bergan T, Ortengren B and Westerlund D. Clinical Pharmacokinetics of Co-Trimazine. Clinical Pharmacokinetics. (1986) 11, 5: 372-386.

52. Sheer M. Studies on the antimicrobial activity of Baytril. Veterinary Medicine Review (1987) 2: 90-99.